Science of the Total Environment 485–486 (2014) 223–231

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Cadmium and other heavy metal concentrations in bovine kidneys in the Republic of Ireland

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HIGHLIGHTS

- An estimated 15% of Irish soils exceed the EU Cd threshold limit of 1 mg/kg.
- 11.3% of cattle had kidney Cd concentrations in excess of the EU ML of 1 mg/kg.
- Age, soil Cd concentrations and region were key predictors of elevated kidney Cd.
- Kidneys of most Irish cattle under three years of age will conform with EU requirements.

ARTICLE INFO

Article history:
Received 17 January 2014
Received in revised form 10 March 2014
Accepted 16 March 2014
Available online 12 April 2014

Editor: F.M. Tack

Keywords:
Cadmium
Heavy metals
Bovine kidneys
Ireland

ABSTRACT

In Ireland, an estimated 15% of Irish soils exceed the EU threshold limit for soil Cd of 1 mg/kg. The aim was to determine the concentrations of Cd and other heavy metals (As, Hg and Pb) in kidneys collected from cattle at slaughter. Systematic sampling of eligible animals (animals that were born and reared until slaughter in the same Irish county) at the time of slaughter was conducted, until a threshold number of animals from all 26 counties and 6 age categories was reached. A predictive surface of soil Cd was generated, by kriging the Cd values of 1310 previously reported soil samples. A linear regression weighted model was developed to model kidney Cd concentration, using the risk factors of age, sex, breed, province and estimated soil Cd concentration. Kidney Cd ($n = 393$) concentrations varied between 0.040 and 8.630 mg/kg wet weight; while concentrations of As, Hg and Pb were low. The estimated weighted proportion of animals with a high (≥ 1 mg/kg) kidney Cd concentration was 11.25% (95% CI: 8.63–14.53%). Key predictors for high kidney Cd concentration were soil Cd, animal age and province. At a soil Cd concentration of 1.5 mg/kg, it was predicted that an age threshold to avoid exceeding a kidney Cd concentration of 1 mg/kg in most animals would be ~3 y in Connacht, >4 y in Ulster, and >5 y in Leinster and Munster. In naturally occurring areas of high Cd levels in soils in Ireland, the Cd level in bovine kidneys can exceed the current EU ML of 1 mg/kg in older animals. Kidneys of most cattle under three years of age will conform with EU requirements.

1. Introduction

Cadmium (Cd) is a naturally occurring heavy metal that is also emitted as part of industrial pollution. It has no known biological function in either animals or humans but mimics the actions of other divalent metals that are essential to diverse biological functions (EFSA Panel on Contaminants in the Food Chain, 2009). Cd is an environmental contaminant that is readily taken up by plants and, through feed intake, is further transferred to animals (Waegeneers et al., 2009a). In animals, Cd is not easily cleared by the cells, and the poor efficiency of cellular export systems explains the long residence time of this element in storage tissues such as the intestine, the liver and the kidneys (EFSA Panel on Contaminants in the Food Chain, 2009). It is for this reason that older animals have higher liver and kidney Cd concentrations (Nriagu et al., 2009), even if the concentrations of Cd in their diets and water are low.

The highest concentrations of Cd in topsoil in Europe occurs in Ireland, England, the western Alps and southern France, the Belgian–

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http://dx.doi.org/10.1016/j.scitotenv.2014.03.065
0048-9697/

German border, south Sardinia, eastern Italy, Slovenia, Croatia, Albania and Greece. High Cd concentrations in most of these areas are the result of intensive agricultural use of phosphate fertilisers and sewage sludge, except in Ireland, southern Sardinia and Poland and the Goslar district in Germany, where naturally occurring high concentrations are found (Pan et al., 2010).

In Europe, maximum limits (ML) for Cd in foodstuffs are set by Commission Regulation No. 1881/2006 (European Communities, 2006), including bovine muscle, liver and kidneys. The regulatory ML for Cd in bovine muscle, liver and kidneys are 0.05, 0.5 and 1.0 mg/kg wet weight, respectively. Several authors have highlighted high kidney Cd concentrations in cattle in contaminated agricultural areas, both in Europe (including Belgium, Waegeneers et al., 2009a,b; the Netherlands, Spierenburg et al., 1988) and elsewhere (Farmer and Farmer, 2000). However, several studies have also highlighted difficulties in agricultural areas of Europe not exposed to industrial contamination (Andersen and Hansen, 1982; Waegeneers et al., 2009a,b; Bilandžić et al., 2010). As an example, the European maximum concentration for Cd in kidneys was exceeded in 47% of kidneys from Belgian cattle raised in uncontaminated areas (Waegeneers et al., 2009b), leading these authors to conclude that the Cd ML in the EU is realistic in Belgium only for cattle up to 2 years of age (Waegeneers et al., 2009a). Commission Regulation No. 1881/2006 also specifies a ML for lead (Pb) in bovine kidneys (0.5 mg/kg wet weight), but not arsenic (As) or mercury (Hg).

In Ireland, it is estimated that 15% of Irish soils exceed the EU threshold limit for soil Cd of 1 mg/kg, particularly in counties Dublin, Meath, Kildare, Westmeath, north Tipperary and Roscommon (Fay et al., 2007), attributable to the chemical composition of limestone in these areas. As yet, however, there is limited understanding of Cd concentrations in Irish cattle at the time of slaughter. The primary objective of this study was to determine the concentration of Cd in kidneys collected from cattle at slaughter in Ireland. In addition, the kidney concentrations of some other important heavy metals (As, Hg and Pb) were investigated.

2. Material and methods

2.1. Animal identification

In Ireland, all bovines are identified at birth in accordance with EU requirements. The Animal Identification and Movement (AIM) system is a centralised database, managed by the Department of Agriculture, Food and the Marine (DAFM), for the identification and movement of all cattle in Ireland. There is significant animal movement within Ireland due to its geography and traditional rearing and trading patterns. Arising from this, many animals will spend their lives on more than one farm and in different geographic regions of Ireland. For this reason, an algorithm was developed for use in AIM to identify cattle eligible for inclusion in the study, being all animals that were born and reared until slaughter in the same county.

Systematic sampling was conducted in participating slaughterhouses, with samples being collected from eligible animals at the time of slaughter, until a predefined number of animals from each county (26 counties) and age category (6 in total) had been reached. The study was undertaken in two phases, focusing on animals >60 months, and on younger cattle (aged 18–60 months). In all counties except Dublin, Kildare and Meath, approximately 12 animals were selected, being approximately 2 animals each in the age categories 18 to <24, 24 to <30, and 30 to <36 months; 1 animal in each in the age categories 36 to <48, and 48 to <60 months, and 4 animals in the age category ≥60 months. In counties Dublin, Kildare and Meath, where a parallel study was being undertaken by DAFM horticulturalists, additional bovine kidney samples were collected, and included in this study. In these three latter counties, a threshold of approximately 40 samples was used, being 8 animals each in the age categories 18 to <24, 24 to <30, and 30 to <36 months; 4 animals in each of the age categories 36 to <48, and 48 to <60 months, and 8 animals in the age category ≥60 months. Samples were collected from 11 participating slaughterhouses in Ireland, from December 2010 to December 2012.

2.2. Sample collection

At each participating slaughterhouse, DAFM personnel notified the Food Business Operator (FBO) when an eligible animal was identified. Both kidneys were removed from each animal with a clean sterile knife and the fat coverings removed. Kidneys were stored in clean plastic bags at −20 °C until sectioning. A laboratory submission form was completed for each animal, and samples were sent to the DAFM Veterinary Public Health Regulatory Laboratory, Backweston Campus, Young's Cross, Celbridge, Co. Kildare, and stored at −20 °C until sectioning.

2.3. Sample analyses

Entire kidneys were allowed to partially thaw. Each kidney was sectioned twice at 90° to the long axis of the kidney, to create a 1 cm wide cross-section of the entire kidney including the renal hilum. The 1 cm wide sections from the left and right kidney from each animal were then combined and sent on ice to the Food and Environmental Research Agency, Sand Hutton, York, United Kingdom for analyses. Samples were homogenised before being digested and analysed using inductively coupled plasma-mass spectrometry (ICP-MS) with collision cell. Reagent blanks and reagent blanks spiked with known amount of each analyte were analysed with test samples for recovery estimate purposes. All results were corrected for reagent blank and spike recovery. The limit of detection was calculated from 3 × standard deviation of reagent blank values adjusted for dilution and sample weight. The limit of quantification was calculated from 10 × standard deviation of reagent blank values adjusted for dilution and sample weight. Heavy metal concentrations are all reported as mg/kg wet weight.

2.4. Data management and analysis

2.4.1. General

Data were managed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Data from the laboratory submission form for each animal was cross-referenced with DAFM's Animal Health Computer System (AHCS) to confirm the animal identity tag, date of slaughter, final herd of residence and that the animal resided within the county boundary throughout its life. Other animal level information was also gathered from AHCS including breed, sex, class, date of birth, herd of origin, number of progeny, number of calving events and movement between herds. Geographic data were generated, managed and analysed using ArcGIS 10.1 (Environmental Systems Research Institute Inc., Redlands, CA, USA).

The location of each animal for mapping and analyses was attributed to the herd in which the animal resided for the longest period in its life, subsequently referred to as the '*max herd*' (n = 357). Herd locations were represented by the centroid of the largest fragment of land associated with each herd according to the Land Parcel Identification System (LPIS) for 2012. The centroid of the herds District Electoral Division (DED) of origin was used to represent the location when LPIS data was unavailable.

Data analyses were conducted using Microsoft Excel and Stata SE version 12.1 (StataCorp, Texas, USA). Where Cd results were below the limit of detection (LOD; 0.01 mg/kg for Cd), we arbitrarily assigned a Cd value of 0.005 mg/kg, being half of the LOD. Maps were created using ArcView GIS 3.2 (Environmental Systems Research Institute, Inc., CA, USA).

2.4.2. Estimating mean soil Cd

Data were available on soil Cd values for 1310 samples throughout Ireland, collected during 1995–96 (295 soil samples) and 2002 (1015 soil samples) by the Environmental Protection Agency (Fay and Zhang,

2013), as mapped by Fay et al., 2007 (Fig. 1). These data were obtained and mapped as point locations. A predictive surface representing soil Cd was generated by kriging these points using ordinary kriging with a Gaussian semivariogram model. A variable search distance with 12 neighbours was selected with an output raster cell size set of 50 m. Kriging is a geostatistical analytical technique which assumes that the distance and direction between sample points relates to an underlying spatial correlation that can be used to explain variation in a predicted surface. In this analysis, kriging was used independently of other variables such as soil type or geology to create a simple mathematical predictive surface based on proximity to neighbouring points and their respective Cd value.

Of the 357 farms sampled for kidney Cd, 349 farms were identified through DAFM's Land Parcel Identification System (LPIS). For spatial analysis at a national level, the centroid of the largest fragment of land of a farm is normally used as a point representation of the farm. For the purposes of this study, however, all the fragments in a farm were represented. For this reason, a point map was created consisting of

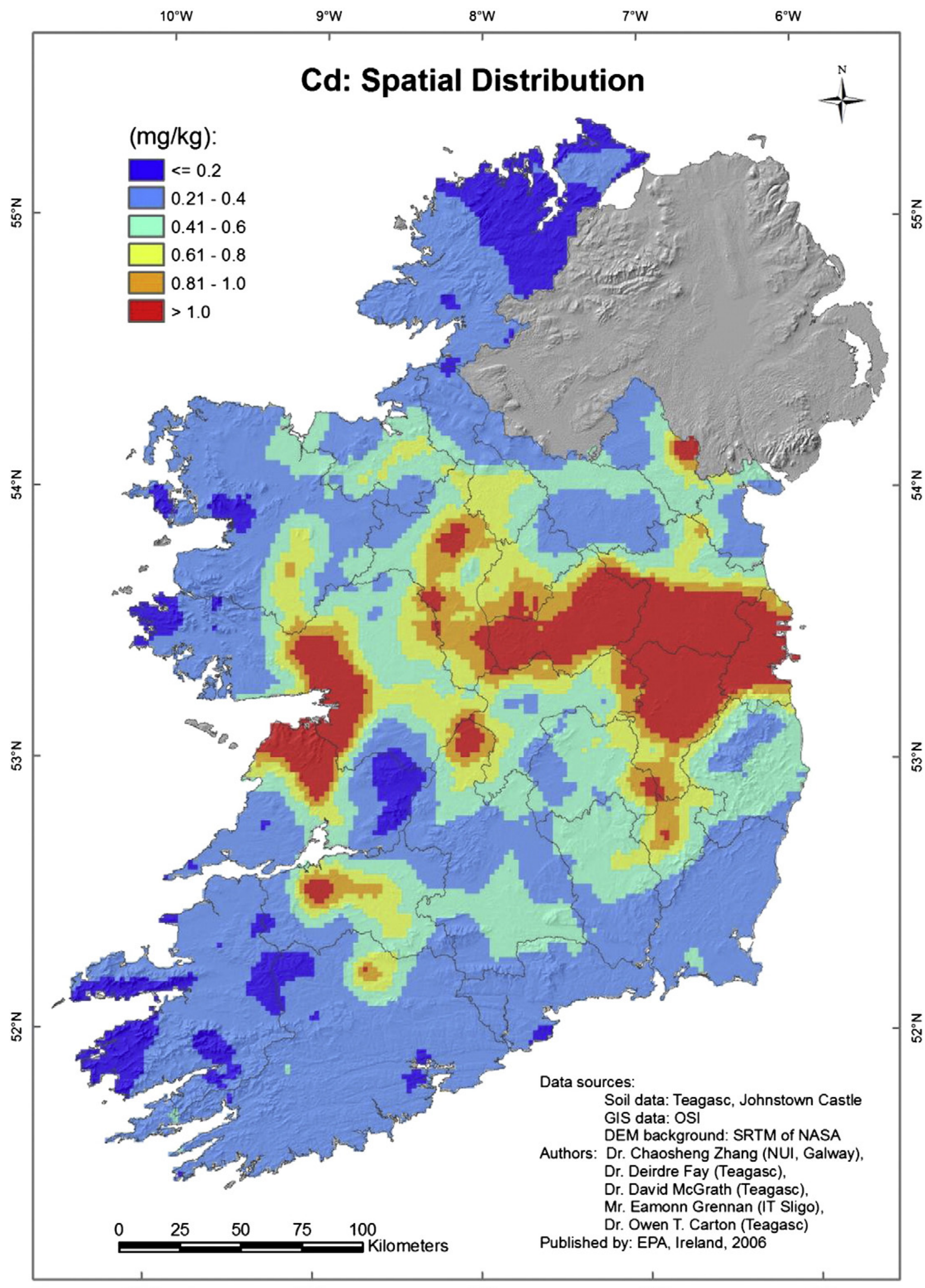

Fig. 1. Estimated soil Cd concentration in Ireland (used with permission, from Fay et al., 2007).

2317 fragments for the 349 farms. The fragment point map was queried to find the value of the underlying kriged Cd surface for each point using a bilinear interpolation of each value. This addresses the issue of a value from a single point representing a fragment of land. Subsequently, an estimated mean, median, minimum and maximum soil Cd concentration was calculated for each of the 349 farms from the kriged Cd concentration assigned to each farm fragment.

2.4.3. Statistical analyses

a. Weighting.

The sample was weighted in order to account for unequal probability of selection within county/age strata. The target population used to calculate weights was 'all animals slaughtered in 2012' (this being the year in which the majority – 67% – of the sample was selected), aged over 18 months. Weights were calculated within each county/age stratum as:

$$N_h/n_h,$$

where N_h was the total number of cattle slaughtered in the stratum in 2012 and n_h was the number of animals sampled in the stratum.

Data were stratified by county. Three of the county/age stratum points had no cattle sampled (Table 1); therefore age groups '36 to <48 months' and '48 to <60 months' were combined so that each stratum contained at least one sampled animal. Each county ($n = 26$) was also assigned to the province ($n = 4$; Ulster, Munster, Leinster and Connacht) in which it is located.

b. Univariable analysis.

The distribution of kidney heavy metal (Cd, As, Hg and Pb) concentrations was estimated, using both unweighted and weighted observations. The weighted and unweighted distribution of Cd concentrations was also estimated by the five risk factors:

- *age*, categorised into 6 groups (18 to <24, 24 to <30, 30 to <36, 36 to <48, 48 to <60, ≥60 months),
- *sex*,
- *breed*, either dairy or beef,
- *province of the* max herd, and
- *estimated median concentration of Cd in the soil of the* max herd, continuous for modelling purposes and grouped into low (<0.555) and high (≥0.555) concentrations, based on the median for descriptive purposes.

c. Multivariable analysis.

A linear regression model was developed to model the concentration of Cd in kidneys, using each of the five described risk factors. Initially a model was developed using the unweighted data. The 'regress' command in STATA SE version 12.1 (StataCorp, Texas, USA) was used to build the unweighted model. Whether to include continuous variables (the median Cd soil concentration and age) as linear was assessed by examining plots of the kidney Cd concentration against each risk factor. An initial model was created by including all risk factors then reducing the model using a backward selection procedure based on the F-test ($p > 0.05$). Residual plots from the initial model were examined to identify whether the dependent or independent variables needed to be transformed. Outliers were also examined in order to assess the fit of the model. The model developed using the unweighted data was then recreated using weighted data. The 'survey regress' command in STATA SE version 12.1 (StataCorp, Texas, USA) was used to model the weighted data and to account for the stratified sample design. County/age stratum points with a single sample unit were centred so that the variance was calculated at the overall variance.

3. Results

3.1. The study animals

There were 393 animals sampled from 357 *max herds*. Of these, 326 *max herds* had only 1 animal sampled, 28 had 2 animals, 2 herds had 3 animals and 1 herd had 5 animals sampled. The median age of the study population was 3.0 years, composed of 69% females and 70% beef breeds.

Table 1
Sample population of cattle slaughtered between December 2010 and December 2012 by province and county of the herd at slaughter, and age category.

Province of herd at slaughter	County of herd at slaughter	Age (months)						Total
		18 to <24	24 to <30	30 to <36	36 to <48	48 to <60	≥60	
Connacht	Galway	2	2	2	1	1	4	12
	Leitrim	3	1	2	1	1	4	12
	Mayo	2	2	2	1	1	4	12
	Roscommon	2	2	2	1	2	4	13
	Sligo	2	2	2	1	1	5	13
Leinster	Carlow	3	3	2	1	1	2	12
	Dublin	3	6	7	2	1	11	30
	Kildare	6	6	6	3	3	4	28
	Kilkenny	2	2	2	1	0	5	12
	Laois	2	3	2	1	1	4	13
	Longford	1	3	2	1	1	3	11
	Louth	2	4	1	2	1	7	17
	Meath	5	6	5	4	3	19	42
	Offaly	2	2	2	1	1	4	12
	Westmeath	2	2	2	1	1	4	12
	Wexford	3	2	3	1	1	4	14
	Wicklow	2	2	2	1	1	5	13
Munster	Clare	2	3	2	1	1	4	13
	Cork	2	2	2	1	1	4	12
	Kerry	2	2	2	1	1	5	13
	Limerick	2	3	2	1	1	4	13
	Tipperary	2	3	3	0	3	4	15
	Waterford	2	2	2	1	1	5	13
Ulster	Cavan	2	2	1	2	1	4	12
	Donegal	2	2	2	1	1	4	12
	Monaghan	2	2	2	0	2	4	12
Total		62	71	64	32	33	131	393

3.2. Univariable analysis

A summary of the concentrations of each of the heavy metals within kidneys, for both weighted and unweighted data is shown in Table 2. The location of *max herds* included in the study and the kidney Cd status of animal in these herds are shown in Fig. 2. Kidney Cd concentration increased significantly ($p < 0.001$) as age and soil Cd increased (Fig. 3). The Spearman correlation coefficient between soil and kidney Cd concentration was 0.42 and between kidney Cd and age was 0.64.

The effect of each of the risk factors on kidney Cd is shown in Table 3 for both weighted and unweighted data. The estimated weighted proportion of animals with a high (≥1 mg/kg) kidney Cd concentration was 11.25% (95% CI: 8.63% to 14.53%).

3.3. Multivariable analysis

Of the 393 animals sampled, 8 did not have any data on the estimated soil Cd concentrations, therefore, the linear model was developed using data from the remaining 385. Initially a linear regression model was built using backward selection and including the risk factors: the natural logarithm (ln) of age and estimated soil Cd concentrations, the province of the *max herd*, breed and sex. Based on the residuals from the initial linear unweighted model, it was necessary to take the natural logarithm of the kidney Cd concentrations. The final unweighted linear model contained the risk factors: natural logarithm of age and soil Cd concentration and the province of the *max herd* (Table 4), the model was significant ($p < 0.001$) and the adjusted R^2 was 61.1%. The same model was then fit using weighted data and accounting for the stratified survey design (Table 4), again the model was significant ($p < 0.001$) and the unadjusted R^2 was 54.9% (note an adjusted R^2 could not be calculated for the weighted model). The coefficients from the weighted and unweighted models were very similar, with the exception of being smaller for Munster and for estimated soil Cd concentrations in the weighted model. Kidney Cd concentrations increased significantly ($p < 0.001$) with both increasing age and estimated soil Cd concentrations, and were significantly higher in Connacht compared to the other Irish provinces (Table 4).

The predicted kidney Cd concentrations based on the weighted model, by age, province of the *max herd* and a range of estimated soil Cd concentrations are shown in Fig. 4. For most provinces and soil concentrations shown, the ML on average is not reached until over 4 years of age. The only exception is cattle in the higher estimated soil Cd concentration (1.5 mg/kg) in Connacht which reached the ML at 3 years of age.

4. Discussion

An estimated 11.3% of the study animals, using weighted proportions, had kidney Cd concentrations exceeding the European ML, with kidney Cd concentrations varying from 0.040 to 8.630 mg/kg wet weight. Three animals had a kidney Cd concentration greater than 4.0 mg/kg: a 13.5 year old cow from Co. Roscommon in Connaught (8.63 mg/kg), a 14 year old cow from Co. Meath in Leinster (5.04 mg/kg) and an 8 year old cow from Co. Tipperary in Munster (4.35 mg/kg). All three animals had remained on their farm of birth until slaughter. These data were substantially right skewed, with most values over 1.0 mg/kg not being substantially higher. To illustrate, the 90th weighted percentile for kidney Cd concentration was 1.08 mg/kg, the 95th weighted percentile was 1.50 mg/kg, and the 99th percentile was 3.52 mg/kg. These results are analogous to recent reports from Belgium, where 47% of cattle from non-contaminated areas had kidney Cd levels in excess of the European ML (Waegeneers et al., 2009a).

The kidney concentrations of the other heavy metals under investigation were very low. Currently, a ML is specified under European legislation for Pb (0.50 mg/kg wet weight for Pb in offals of bovine animals; European Communities, 2006), but not for either As or Hg. The median kidney Pb concentration was 0.03 mg/kg (in comparison to the ML of 0.5 mg/kg), with no samples exceeding the ML. This information about kidney As and Hg concentrations in Irish cattle will provide valuable background information, if a ML for these heavy metals is introduced into the EU at a future date.

In the final multivariable model, kidney Cd concentrations were significantly associated with the ln of estimated soil Cd concentration, the ln of age and province (Table 4). Each will be considered in turn. It is important to note that Irish beef and dairy production systems are primarily pasture-based. Although animals may receive some feed supplements, the principal source of nutrition of bovines in Ireland is by grazing or consuming conserved grasses. There is an increasing understanding of mechanisms of Cd intake in farmed animals, and the conditional association between estimated soil and kidney Cd concentrations, as identified in this study, is likely a reflection of increased Cd exposure with increased soil Cd concentrations, presumably through intestinal absorption. Intestinal absorption is influenced by the type of diet and nutritional status of the animal involved (IPCS, 1992), with bioavailability increased, in rats at least, with even marginal dietary deficiencies of Fe, Zn and Ca (Reeves and Chaney, 2002, 2004). In Ireland, soil Cd concentrations are not homogeneous, but at higher concentrations in some areas of counties Meath, Kildare, Dublin, Westmeath, Galway, Clare Limerick, Roscommon and Offaly. In this study, the estimated median soil Cd concentration at each *max herd* varied between 0.13 and 2.32 mg/kg, with 95% below 1.5 mg/kg. For comparison, there are areas of Europe where the Cd concentrations in top soils are both lower and higher (range: 0.01 to 14.1 mg/kg). In general, however, the estimated Cd concentrations in Ireland (median 0.57 mg/kg; mean 0.69 mg/kg) are substantially higher than the median (0.145 mg/kg) and mean (0.284 mg/kg) values reported throughout Europe (Pan et al., 2010). The general increase in kidney Cd concentration in Irish cattle with age was expected, consistent with bioaccumulation of Cd with time (Waegeneers et al., 2009a). The association between age and kidney Cd concentration in livestock is very well described. For example, cattle

Table 2
Distribution of concentrations of Cd, As, Hg and Pb in 393 bovine kidneys in Ireland during December 2010 to December 2012.

Heavy metal	Kidney concentration (mg/kg wet weight)					
	Minimum	Maximum	Mean	Lower quartile	Median	Upper quartile
Unweighted data						
Cadmium	0.040	8.630	0.734	0.240	0.520	0.890
Arsenic	0.010	0.130	0.019	0.010	0.010	0.020
Mercury	0.003	0.230	0.009	0.003	0.007	0.010
Lead	0.005	0.280	0.048	0.020	0.030	0.060
Weighted data						
Cadmium	0.040	8.630	0.487	0.170	0.280	0.550
Arsenic	0.010	0.130	0.020	0.010	0.010	0.020
Mercury	0.003	0.230	0.008	0.003	0.007	0.010
Lead	0.005	0.280	0.045	0.020	0.030	0.050

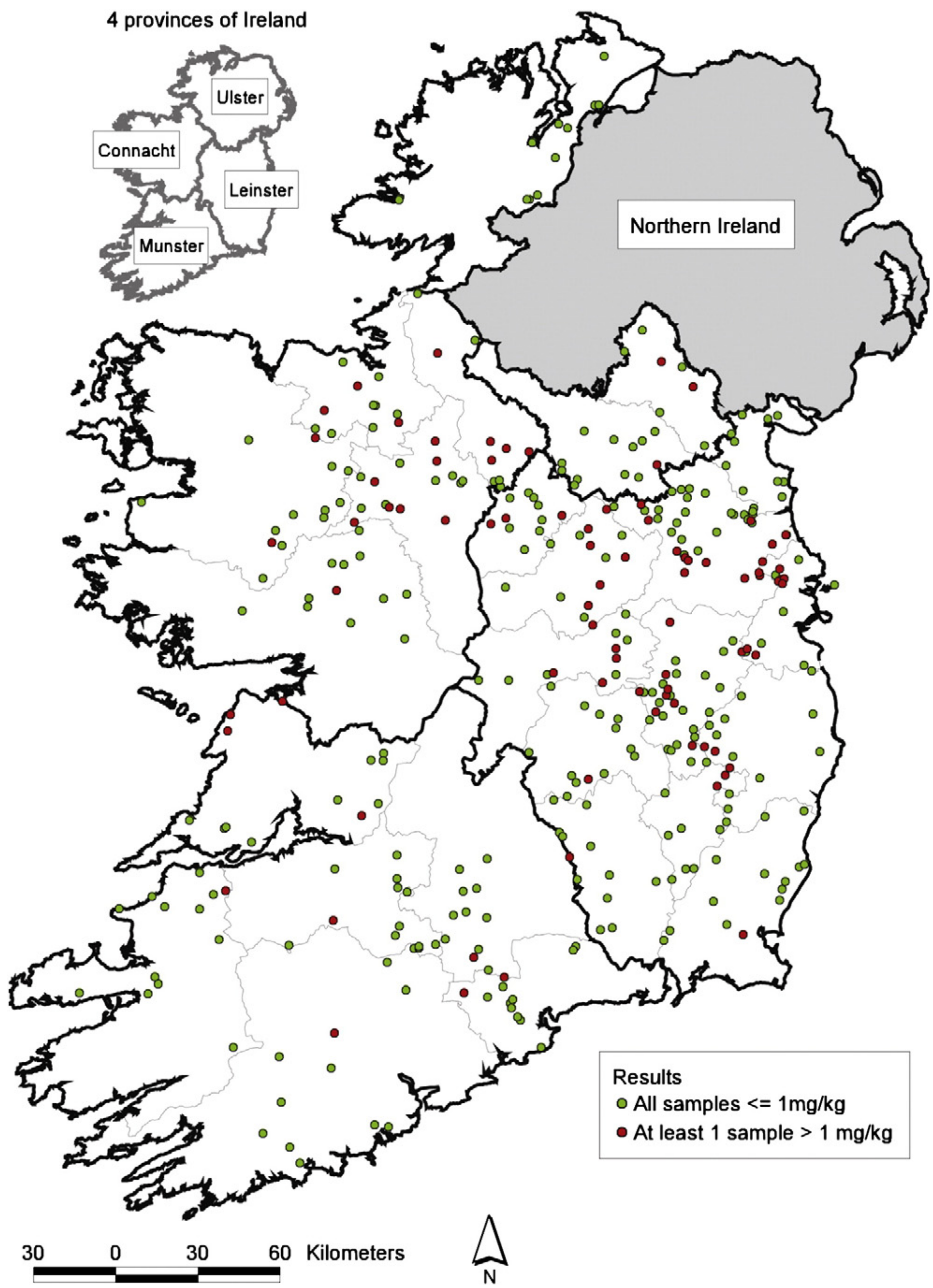

Fig. 2. Location of 357 *max herds*, and kidney Cd concentrations (mg/kg wet weight; all samples ≤1 mg/kg, green dot; at least 1 sample >1 mg/kg, red dot) of 393 bovine animals slaughtered between December 2010 and December 2012, in each of the four provinces (Ulster, Munster, Leinster and Connacht) of Ireland.

from north eastern Poland aged between 8 and 12 years had kidney cortex Cd concentrations between 0.68 and 2.0 mg/kg wet weight. Free ranging bison from the same area, aged 7 to 12 years, had kidney cortex concentrations between 1.95 and 3.52 mg/kg wet weight (Włostowski et al., 2006). Similarly in northwestern Spain, cows had significantly higher kidney Cd concentrations than female calves (López Alonso et al., 2000). In Jamaica, where soil Cd concentrations are high, cattle kidney Cd concentrations between 0.012 and 117 mg/kg have been reported, with the Cd contents of older cows much higher than those of younger animals (Nriagu et al., 2009). The influence of province was significant, after accounting for both estimated soil Cd concentration and animal age. In particular, after accounting for these other two variables, animals sampled in Connacht had the highest median concentrations of kidney Cd. We can currently only speculate on reasons for this difference. It could also, in part, be an artefact of the sample design since the sample size was much smaller in Connacht (62) compared to Leinster (215). Further, the area of high soil Cd passes through certain counties in Connacht, and it is possible, given the limited sample size in this province, that the estimated soil Cd concentrations is an imprecise measure of the actual value near the farms in the survey. It may also relate to other factors affecting Cd absorption, including differences between provinces in terms of trace element status.

In Fig. 4, we present predicted kidney Cd concentrations based on the weighted linear regression model. The key predictors in the weighted model were soil Cd, animal age and province, and therefore there is a need for different interpretations by province, when considering kidney Cd concentrations by age and soil Cd. We illustrate using a soil Cd concentration of 1.5 mg/kg (the 95th percentile for estimated soil

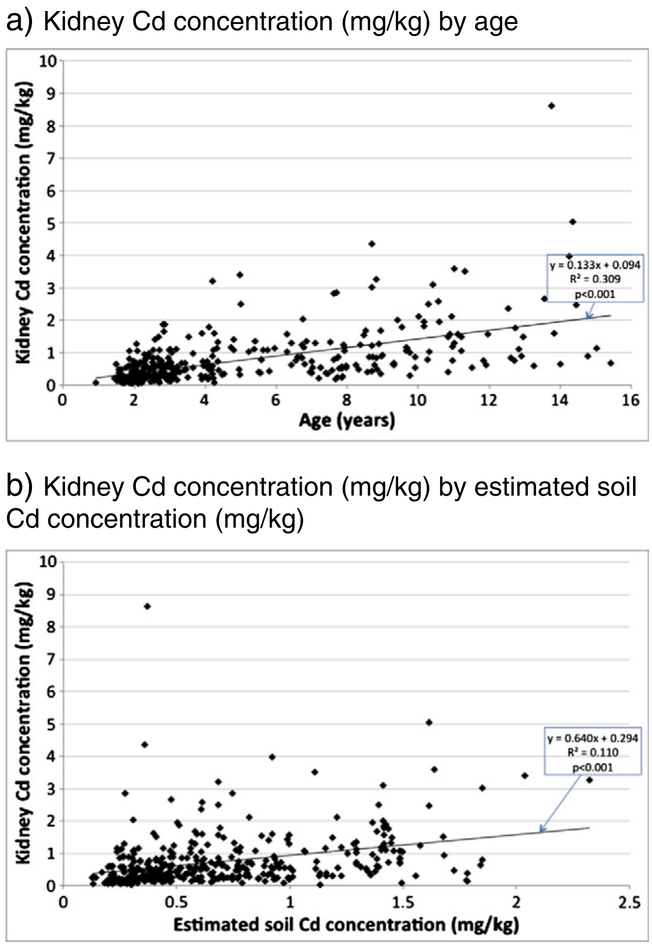

Fig. 3. Scatter plots of the kidney Cd concentrations (mg/kg wet weight; in 393 cattle slaughtered between December 2010 and December 2012 by a) age (in years) or b) estimated Cd soil concentrations (in mg/kg; 385 study cattle) in Ireland.

Table 4
Parameter estimates from the unweighted and weighted linear regression model of the natural log (ln) concentration of cadmium (mg/kg wet weight) in the kidney of 385 cattle slaughtered between December 2010 and December 2012 in Ireland.

Risk factor	Estimate	S.E.	95% C.I.		p-Value
			Low	Upper	
Unweighted					
Intercept	−6.46	0.34	−7.12	−5.79	
Ln soil Cd concentration	0.67	0.06	0.56	0.79	<0.001
Ln age	0.90	0.05	0.81	0.99	<0.001
Province					
Leinster	−0.54	0.09	−0.71	−0.37	<0.001
Munster	−0.45	0.11	−0.66	−0.24	<0.001
Ulster	−0.42	0.13	−0.67	−0.17	0.001
Weighted					
Intercept	−6.80	0.60	−7.98	−5.63	<0.001
Ln soil Cd concentration	0.60	0.09	0.42	0.78	<0.001
Ln age	0.94	0.08	0.77	1.10	<0.001
Province					
Leinster	−0.52	0.09	−0.69	−0.34	<0.001
Munster	−0.56	0.13	−0.82	−0.30	<0.001
Ulster	−0.38	0.12	−0.61	−0.14	0.002

Cd concentration on *max herds* in this study was 1.48 mg/kg). At this value, an age threshold to avoid exceeding a kidney Cd concentration of 1 mg/kg in most animals would be ~3 y in Connacht, >4 y in Ulster, and >5 y in Leinster & Munster. A similar predictive approach has been used previously, particularly in Belgium, where calculations for 2-year-old animals from non-contaminated areas showed that in this group the European ML for Cd in kidneys would be exceeded in zero to 5% of cases (Waegeneers et al., 2009a).

We highlight a range of issues with the potential for impact on study interpretation. With respect to sample collection, study animals were identified using random sampling methods. We have no reason to believe that these cattle are unrepresentative of the broader reference population of Irish cattle at slaughter. Further, samples for analysis were collected from representative cross sections of the kidney, thus including kidney cortex and medulla, consistent with recommendations from Roggeman et al. (2014) that whole organ mixing is not necessary. As highlighted previously, several counties (Dublin, Kildare, Meath) were substantially overrepresented with respect to the number of samples that were collected in those counties, for reasons explained previously. However, this was taken into account through the use of weighting in the analyses. Weighting was conducted using all slaughtered animals in 2012 as the target population. The results should therefore be representative of the slaughtered animal population and

Table 3
Distribution of kidney Cd concentrations (mg/kg wet weight) in cattle slaughtered between December 2010 and December 2012 in Ireland, by categorised risk factors.

Risk factor	Number of animals	Unweighted data			Weighted data		
		Lower quartile	Median	Upper quartile	Lower quartile	Median	Upper quartile
Age (months)							
18 to <24	63	0.14	0.22	0.38	0.13	0.18	0.29
24 to <30	71	0.18	0.28	0.52	0.12	0.26	0.35
30 to <36	64	0.24	0.38	0.67	0.17	0.28	0.59
36 to <48	31	0.29	0.50	0.69	0.24	0.45	0.55
48 to <60	33	0.32	0.55	1.09	0.22	0.47	0.72
≥60	131	0.61	1.00	1.56	0.41	0.78	1.25
Sex							
Female	273	0.30	0.59	1.13	0.21	0.35	0.72
Male	120	0.17	0.28	0.56	0.13	0.21	0.35
Province							
Connacht	62	0.43	0.60	1.16	0.35	0.52	0.75
Leinster	215	0.27	0.55	1.04	0.18	0.29	0.66
Munster	79	0.18	0.28	0.59	0.15	0.21	0.35
Ulster	37	0.22	0.32	0.61	0.14	0.24	0.39
Breed							
Beef	275	0.23	0.49	0.86	0.16	0.28	0.54
Dairy	118	0.25	0.54	1.03	0.18	0.27	0.59
Estimated soil Cd concentration (mg/kg)							
Low (<0.555)	186	0.21	0.33	0.60	0.15	0.23	0.40
High (>=0.555)	199	0.38	0.66	1.21	0.24	0.47	0.78
Missing	8	0.19	0.40	0.81	0.20	0.33	0.75

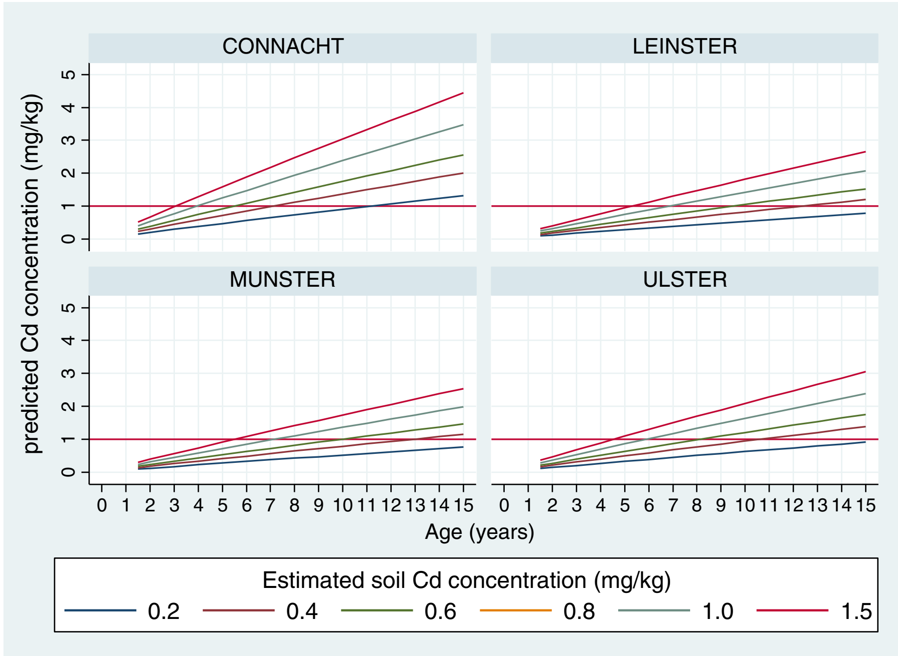

Fig. 4. Predicted kidney Cd concentrations (mg/kg wet weight) based on a weighted linear regression model. Predictions are made by age, province (as determined by the location of the *max herd*, the herd of maximum residence) and estimated soil Cd concentration. The horizontal line indicates the maximum level (ML) for Cd in kidneys (1.0 mg/kg).

applicable when predicting concentrations of Cd likely to be present at the time of entering the food chain. Since the number sampled in some of the strata was small, this resulted in some large weights (ranging from 215 to 27,301). De Leeuw et al. (2008, page 337) describe a measure to determine the increase in variance due to weight variation termed 'Unequal Weighting Effect (UWE)', which indicates whether or not the weights are too extreme and if they may need trimming. This is calculated as $1 + cv^2$, where cv is the coefficient of variance of the weights and calculated by the standard deviation of the weights divided by the average weight. In this sample, the cv was $3903 / 3574 = 1.09$ and the overall UWE for this survey was 2.35, which suggests that there was very little inflation and that it was not necessary to trim the weights (De Leeuw states that a large UWE would be greater than five). In the analysis, clustering was not accounted for since the majority of herds (326 out of 357) had only a single animal sampled. Also, during data analysis, no investigation was conducted of the potential association between kidney Cd concentrations with proximity of animals to large urban areas, potential sources of industrial contaminants or agricultural sources of Cd, such as animal feed and fertiliser.

5. Conclusions

This study suggests that bovine kidney Pb concentrations are unlikely to exceed EU guidelines in cattle reared and slaughtered in Ireland. However, Ireland has naturally occurring areas of high soil Cd concentrations. The estimated 11.3% of cattle slaughtered in Ireland have kidney Cd concentrations in excess of the EU ML of 1 mg/kg. Age, soil Cd concentrations and region were key predictors of kidney Cd concentrations. In areas with Cd soil concentrations of 1.5 mg/kg, an age threshold to avoid exceeding a kidney Cd concentration of 1 mg/kg in most animals would be ~3 y in Connacht, >4 y in Ulster, and >5 y in Leinster & Munster. In naturally occurring areas of high Cd levels in soils in Ireland, the Cd level in bovine kidneys can exceed the current EU ML of 1 mg/kg in older animals that have had longer lifetime exposure. This study estimates that the kidneys of most prime cattle and all cattle under three years of age will conform with EU requirements.

Conflict of interest

The authors declare no conflict of interest.

Role of the funding source

The study was funded by the Irish Department of Agriculture, Food and the Marine. Several co-authors are DAFM employees, including the senior author.

Acknowledgements

We wish to thank DAFM staff in slaughterhouses with their assistance in sample collection, Richard O'Keefe for his assistance with sample collection and storage, and Brian Cloak and Dr. Marijke Beltman for their assistance with sample processing.

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